

Freshwater Algae Chara L. Species Identification by Molecular Phylogenetics

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Research Article

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Abstract

The study focused on molecular phylogeny to identify *Chara* species using the *rbcl* genes. *Chara* species were found in both stream and brackish waters in Tirumala and Talakona, Andhra Pradesh, India. *Chara* DNA was isolated with DNeasy plant Mini Kit (Quiagen, Hilden, Germany). For quality assessment of isolated DNA 1.0% agarose gel were used. *Chara* species were identified using BLAST with the NCBI GenBank database's 'nr' database, Clustal W multiple alignment tools, and MEGA6 software. The *Chara* species identified as *Chara zeylanica* based on the highest identity score. This study is a pioneering attempt at molecular phylogeny for the Charophyceae of India.

INTRODUCTION

Class Charophyceae characteristics with macroscopic algae habitat in brook and briny water, the plant body shows great elaboration in their structure (Subramanian, 2018). It is erect, long, slender green main axis with nodes and internodes. The branching axis is central, each node arises a whorl of lateral branches. Sexual reproduction Oogamous and complex in Charophyceae (Vashishta, 2020). In India the family Characeae reported 5 genera i.e. *Chara*, *Nitella*, *Lychnothamnus*, *Nitella* and *Tolypella*. The Genus *Chara* reported about 27 species from India (Gupta, 2012).

Chara is small, simple thalli compare land plants and ease manipulation and close relation to the land plant make them attractive research models in Plant physiology, Molecular biology, biochemistry and cell cycle (David *et al*, 2016; Rekha *et al* 2023). Charophyceae algae improve the water quality, control nutrient cycle, provide the food for fish and aquatic birds so, they have ecological value. So, this Charophyceae have the importance in ecological and economical context (Barbosa *et al* 2021, Rekha *et al* 2023). Availability of taxonomical details, diversity and occurrence is important for upcoming research work (Rekha *et al* 2023).

Identification of algae and evolutionary relationships are studies under Systematics (Braun, 1834). Identification algae done by both morphological and molecular phylogenetic studies (Akash *et al* 2018). In the genome short gene in uniform locality used for the molecular phylogenetic identification of the algae. DNA barcode of each alga is unlike, so molecular phylogenetic studies have importance in taxonomical and evolution studies (Alberts *et al*, 2002).

In some algae similar structure of organism also shows different molecular phylogenetic studies (Lorenz, Todd, 2012). In morphological method *Chara* species without sex organs most of the cases identification not possible (Subramanian, 2018). Molecular phylogenetic technique increased accurate identification organism, help in the effective nomenclature (Nair *et al*, 2024).

MATERIAL AND METHODS

Sample Collection

The samples are collected from water resources of Talakona and Tirumala, Andhra Pradesh, India. Samples are collected three seasons in the study area about 10 different ponds, ditches and they named them as CH1 to CH20. The *Chara* samples are collected with soil and water where they grow. The samples are collected with soil and water where they grow and cultured for 75 days in vivo condition. The samples were separated from other algae, thoroughly washed with the water (Zulkarnain *et al*, 2016).

Molecular Phylogenetic

Chara DNA was isolated with DNeasy plant Mini Kit (Quiagen, Hilden, Germany). For quality assessment of isolated DNA 1.0% agarose gel were used. To Amplifying the fragments of rbcL gene primers rbcL-F and rbcL-R (Marit Mjelde *et al* 2021) were used. DNA sequencing forward and reverse reaction of PCR amplicon carried out with rbcL-F and rbcL-R primers. To carry out this reaction BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer used for removing impurities. PCR conditions at 1cycle 94⁰c for 3 min, 30 cycles for 94⁰ for 1 min, 55⁰ c for 40s, 72⁰ c for 55 s and 1 cycle for 72⁰ c for 7 min were maintained.

Online pairwise alignment tool software used for generating consensus sequence of rbcL gene done by forward and reverse sequence. To find the Similarity sequence BLAST with the 'nr' database of NCBI GenBank database. Clustal W software used for alignment and phylogenetic tree constricted with help of MEGA6 Software.

RESULTS AND DISCUSSION

Morphological Characters This *Chara* species are robust, grow 750 to 780 µm in width, and 6- 10 branchlet in each whorl. Bracts and bracteole are short and stipulodes occur in 2 rows. Internodes grow 1 ½ time longer than branchlet.

Collection number: Talkona surrounding areas and Number: 018

Distribution: First report from Southern India (Gupta, 2012)

Molecular Phylogenetic

The CH2 sample agarose gel analysis revealed a single band of high molecular weight of a high-molecular DNA. A Single distinct 690bp PCR amplicon was identified. rbcL genes were generated and used for BLAST through the 'nr' database of NCBI GenBank. Ten sequences were selected, aligned, and the phylogenetic tree was built according to the identity score. The CH2 sample shows 99.64% homology to the *Chara zeylanica*. The Ident of the Sample shows highly similar to the *Chara* sp. NIBRCR0000100094 but query cover shows only 98%. Whereas *Chara zeylanica* shows 99% query cover and 99.65% Ident, so Ch2 sample were Identified as *Chara zeylanica*.

Table: 1. Sequences alignments for homology

Sequences producing significant alignments					Algae Identification		
S.No	Description	Max Score	Total score	Query cover	E value	Ident	Accession
1.	<i>Chara zeylanica</i>	1040	1040	99%	0	99.65%	AY720934.1
2.	<i>Chara</i> sp. NIBRCR0000100094	1038	1038	98%	0	100.00%	KT343914.1
3.	<i>Chara zeylanica</i> isolate Seosan 1	1038	1038	98%	0	100.00%	KT343913.1
4.	<i>Chara zeylanica</i>	1037	1037	99%	0	99.65%	DQ076299.1
5.	<i>Chara zeylanica</i> strain KGK0057b	1035	1035	98%	0	100.00%	HQ380468.1
6.	<i>Chara haitensis</i> isolate KGK0199	1031	1031	98%	0	99.82%	KX431011.1
7.	<i>Chara zeylanica</i> strain KGK0403	1029	1029	98%	0	99.82%	HQ380476.1
8.	<i>Chara kenoyeri</i> strain TP118	1029	1029	98%	0	99.82%	HQ380465.1
9.	<i>Chara hydropitys</i> strain KGK0213	1029	1029	98%	0	99.82%	HQ380461.1
10.	<i>Chara haitensis</i> strain KGK0483	1029	1029	98%	0	99.82%	HQ380455.1

Phylogenetic Tree

DISCUSSION

Taxonomical investigations based on morphology may be result in misidentification (Karan et al, 2017). To avoid this, molecular approaches have been employed. The 18s RNA, matK, ITS2, and rbcL genes are frequently used for molecular phylogenetic analysis of plants and algae (Prakash and Sanjay, 2018).

Charophyceae are subaquatic meadows that grow in relatively quiet, clean, fresh or brackish waters. The algal thallus has a very elaborate structure. Thallus are *Chara* erect, long, slender, green or grey main axis with nodes and internodes.

The occurrence of morphological plasticity makes it challenging to recognize *Chara* members. The phenotypic plasticity is happened by the environmental changes and to overcome the phenotypic plasticity, DNA barcodes (ITS2, Mat K and rbcL) areas are useful to identify the *Chara* species (Schneider *et al*, 2015).

Chara samples of about 20 are collected in the Tirumala and Talakona areas. These samples are then analyzed to assess their genetic diversity and ecological adaptations. The samples are morphologically dissimilar from different ponds and ditches. Some are stout, some are long, some are light green and thick green. Few samples show the reproductive parts, the remaining are without reproductive parts collected in the three seasons. Without sexual organs, the identification is difficult. Due to morphological plasticity and the absence of sexual organs in these samples, I have carried out molecular phylogenetics.

MC Court *et al.* (1996) used eleven morphological characters and *rbcl* sequence data to reconstruct the phylogeny of the genus *Chara*. Monique Turmel *et al.* (2006) investigated the *atpB*, *rbcl*, 18S rRNA, and mitochondria of *Chara vulgaris* and concluded that Charales stay sister to the green plants of land. Hidetoshi (2009) identified *Chara globularis* using morphological and molecular phylogeny based on *rbcl* gene sequencing. Schneider *et al.* (2015) studied the *rbcl*, ITS2, SNP, *MATK*, and 18s rRNA markers used to identify species divergence in the *Chara* genus. Jacek and Michal (2017) investigated the *atpB*, *matK*, and *rbcl* sequences of *Chara globularis* var. *tenuispina* and determined that it is a distinct species from *Chara globularis*.

The *rbcl* markers greatly rises the cost of sequencing the locus of barcode, it ideal for the Charophytes species level variability and sometime this marker alone not ideal for the identification (Hall,2010). The *rbcl* genes shares mutual codon for closely related species (Akash *et al*, 2018). So, *rbcl* based technologies can enhances efficacy of the algae identification and evolutionary relationship (Pheravut and Ywadee, 2014). These studies supported that *rbcl* genes and markers revealed the exact identification of *Chara* species through molecular phylogenetics. However, both molecular and morphological data together perfect for identification, when it separates this tool are not ideal (Sakayama 2008).

From India total 10 sequence of *Chara Zeylanica* are in the NCBI, which are identified with Maturase K genes 541 bp linear DNA (Balakrishnan and Rani, 2020). This study is a pioneering attempt at molecular phylogeny for the Charophyceae of India.

CONCLUSION

Chara species have been identified as *Chara zeylanica* based on molecular phylogenetic studies. To identify the *rbcl* gene sequence, BLAST was performed against the NCBI GenBank 'nr' database. The first 10 sequences were selected based on the highest identity score and then aligned using the multiple alignment software application Clustal W. MEGA6 was used to generate the phylogenetic tree. This study is a pioneering effort at molecular phylogeny for the Charophyceae of India.

Declarations

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contribution

"Dr. Rekha Adimulam" corresponding author- wrote the conducted and wrote the manuscript. Prof P. Sujathamma guided and reviewed the manuscript

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Figures



Figure 1

Chara zeylanica 160Pixel =4.23cm

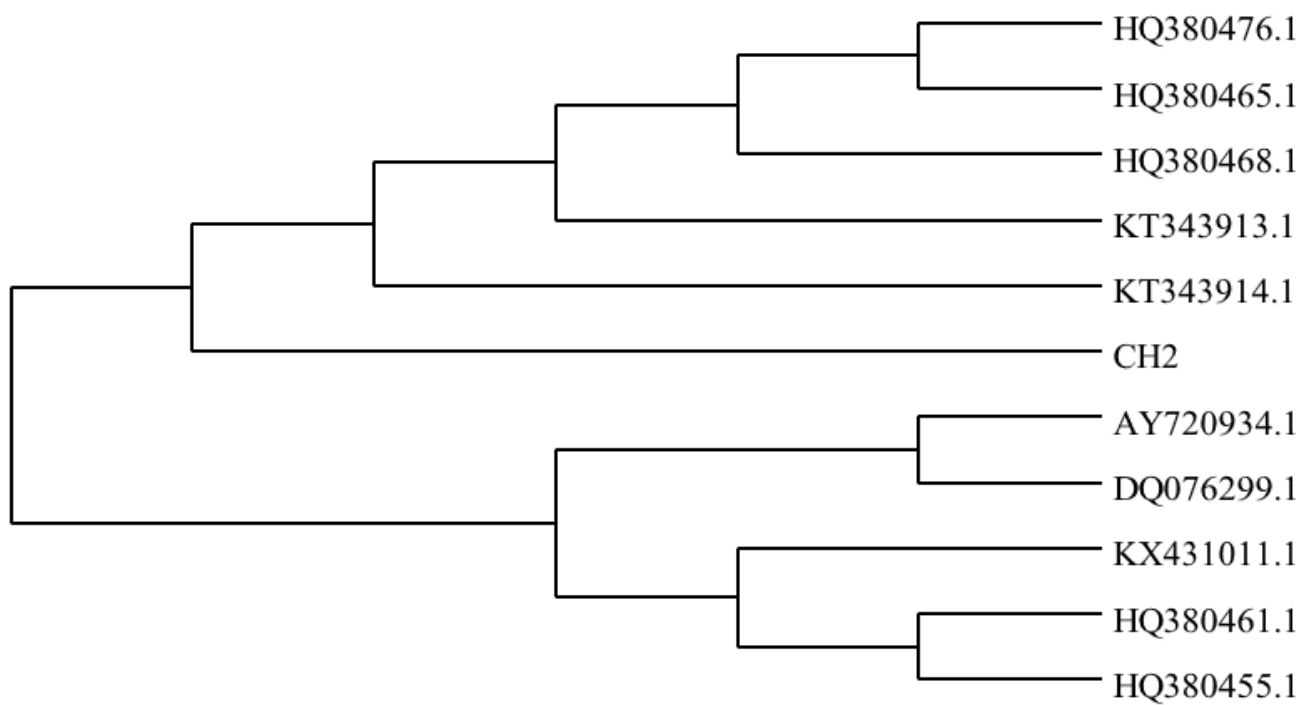


Figure 2

Molecular Phylogenetic analysis by Maximum Likelihood method.